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Declaration under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **AMPLICON MELTING ANALYSIS WITH SATURATION DYES**

(57) Abstract: Methods are provided for nucleic acid analysis wherein a target nucleic acid that is at least partially double stranded is mixed with a dsDNA binding dye having a percent saturation of at least 50% to form a mixture. In one embodiment, the nucleic acid is amplified in the presence of the dsDNA binding dye, and in another embodiment a melting curve is generated for the target nucleic acid by measuring fluorescence from the dsDNA binding dye as the mixture is heated. Dyes for use in nucleic acid analysis and methods for making dyes are also provided.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/33429

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68; C12P 19/34; C07D 279/16
 US CL : 435/6, 91.2; 544/51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 435/6, 91.2; 544/51

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	RIRIE, K. M. Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Anal. Biochem., 1997, Vol. 245, pages 154-160. entire document, especially page 154.	1, 14, 16, 24-28, 31, 45-48 ----- 2-8, 13, 15, 17-23, 29-30, 32
Y	US 6,506,568 B2 (SHRIVER et al.) 14 January 2003, (14.01.2003), entire document, especially column 3, lines 20-67, column 4, lines 1-26	1-8, 13-28, 31-37, 39-48, 63-71, 74-75
Y	US 5,658,751 A (YUE et al) 19 August 1997 (19.8.1997), entire document	9-12, 49-69, 72-82
Y	US 6,437,141 B2 (RANDALL et al) 20 August 2002 (20.8.2002), entire document	9-12, 49-62, 72-82
X --- Y	US 6,174,670 B1 (WITTEWER et al.) 16 January 2001 (16.1.2001), entire document, especially, column 11, lines 10-38, column 22, lines 54-64,	1, 5-7, 13-14, 17-28, 30-33, 39-44 ----- 34-38, 45-47, 63-71

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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 "&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

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Continuation of B. FIELDS SEARCHED Item 3:
Biosis, Embase, Caplus, Medline, Lifesci, EAST databases
search terms: LCGreen, cyanine dyes, PCR, SYBR Green